

Original Research Article

Potential of entomopathogenic fungi as biological control agents of whitefly (*Bemisia tabaci* biotype B) (Genn.) (Hemiptera: Aleyrodidae)

ABSTRACT

The efficiency of isolates from entomopathogenic fungus *Beauveria bassiana*, *Lecanicillium muscarium* and *Metarhizium rileyi* in the control of eggs and third-instar nymphs of whitefly, *Bemisia tabaci* biotype B, was evaluated by pathogenicity and virulence tests, under laboratory conditions. For the pathogenicity tests, five leaflets of bean (*Phaseolus vulgaris*) plants containing 20 nymphs or 20 eggs of *B. tabaci* biotype B were used. The leaflets were immersed in suspension containing a concentration of 10^8 conidia/ml of each fungus isolate. Nymph mortality and egg viability were evaluated after seven days. The isolates that caused nymph mortality and egg viability above 80% were submitted to virulence tests, in order to estimate the lethal concentration (LC_{50}) of each isolate. All isolates were pathogenic to eggs and third-instar nymphs of *B. tabaci* Biotype B. JAB07 of *B. bassiana* and LCMAP3790 of *L. muscarium* isolates presented the best results, with nymphs mortality of $96.68 \pm 2.25\%$ and $97.74 \pm 1.56\%$, respectively. Additionally, JAB07 was the most virulent isolate, both for eggs and third-instar nymphs, with LC_{50} estimated of 0.012 and 0.006×10^3 conidia/ml, respectively. We suggest, future field trials are required in order to analyze the real efficiency of this isolate in the control of eggs and nymphs of *B. tabaci* biotype B in field conditions.

Keywords: Microbial control, pathogenicity, virulence, *Beauveria bassiana*, JAB07

1. INTRODUCTION

The whitefly, *Bemisia tabaci* (Genn.) (Hemiptera: Aleyrodidae), is one of the most important agricultural pests worldwide, its species has more than 600 host plant species, such as soybean, bean, cotton, tomato, among others [1, 2]. *B. tabaci* biotype B is the most important strain of *B. tabaci*, and distributed throughout Brazil causing economic estimated at 2 billion dollars in large-scale crops annually [3]. The duration of the cycle life can varies according species, with temperature being one of the most determinant factors. *B. tabaci* life cycle lasts approximately 19 days at 32 °C, and can reach 73 days at 15 °C. Under favorable conditions, this pest can reach 11 up to 15 generations per year and each female being able to oviposit up to 300 eggs during its life cycle (Mansaray & Sundufu, 2009). The immature adult phases cause direct damage to the plant, by suctioning. Furthermore, an indirect damage related to the transmission of more than 100 species of plant viruses [4, 5, 6].

Comment [MJ1]: How does it differ from other strains?

Comment [MJ2]: This should be a number

Comment [MJ3]: Check grammar of this sentence

The control of *B. tabaci* biotype B is performed primarily with chemical insecticides, which has increased the selection of resistant populations of this pest worldwide, as well as problems related to human health and environmental contamination [7]. Thus, integrated programs management using alternative control that do not cause risks to natural enemies, are harmless to humans and environmentally friendly are of great importance.

The biological control of whitefly using entomopathogenic fungi has increased worldwide [8]. These fungi have the unique ability to infect their hosts directly through the integument and have important role in the pest population regulation in the field. The entomopathogenic fungi that have potential for whitefly control include *Isaria fumosorena*, Wize (1904), *Beauveria bassiana* (Bals.-Criv.) Vuill. (1912), *Metarhizium anisopliae* (Metchnikoff) Sorokin (1883), *Lecanicillium* spp. and *Aschersonia* spp. [13, 9]

However, several studies have reported low control efficiency of this pest (*Bemisia tabaci* biotype B) [10, 11, 12]. Thus, research for highly virulent fungi isolates is essential for the success of strategies for the whitefly control, as well as the evaluation of different isolates of entomopathogenic fungi that are used for the control of other pest species. Several fungi species are used as biological control agent of lepidopteran pests, such as *Metarhizium rileyi* (Farlow), *Beauveria bassiana* and *Lecanicillium muscarium*, an important biological control agent of lepidopteran pests. Meantime, in this work, we also evaluated the insecticide activity against whitefly. The objective of this work was to evaluate, in laboratory conditions, the efficiency of *B. bassiana*, *L. muscarium* and *M. rileyi* new isolates for the control of eggs and nymphs of *B. tabaci* biotype B.

2. MATERIAL AND METHODS

B. tabaci biotype B population

The population of *B. tabaci* biotype B was obtained in cabbage plants (*Brassica oleracea* L. var. Acephala) in experimental field of the São Paulo State University, School of Agricultural and Veterinarian Sciences (UNESP-FCAV), Jaboticabal, São Paulo, Brazil. Species identification was performed in the Department of Entomology of the Agronomic Institute of Campinas (IAC), Campinas, São Paulo, Brazil, and was identified as biotype B. Insects were reared in acclimatized room (25 ± 2 °C, $70 \pm 10\%$ RH and 12/12 h of photophase) in bean plants (*Phaseolus vulgaris* L. cv. Pearl), maintaining for 30-days whitefly cycle [13].

Entomopathogenic fungi isolates

The isolates of *B. bassiana* (IBCB18, IBCB35, IBCB66 and JAB07), *L. muscarium* (LCMAP3790) and *M. rileyi* (NOM1950) were obtained from the entomopathogen bank of the Laboratory of Microbial Control of Arthropods Pests (LCMAP). Isolates from *L. muscarium* and *M. rileyi* were maintained in Petri dishes, containing culture medium Sabouraud Dextrose Agar with Yeast Extract (SDAY). The isolates of *B. bassiana* were maintained in Sabouraud Dextrose Agar (SDA) culture medium, incubated in a BOD at 28 ± 1 °C, $70 \pm 10\%$ RH and 12/12h of photophase for eight days. Subsequently, a suspension (10 ml of distilled water + 0.05 of Tween® and entomopathogenic fungus) was mixed, and two serial dilutions were performed to quantify the number of conidia/ml in Neubauer chamber, and standardization at the concentration of 10^8 conidia/ml.

Pathogenicity bioassays with *B. tabaci* nymphs

Bean plants (*P. vulgaris* L. cv. Pearl) were transferred to a rearing cage 25 days after emergence of the plants. *B. tabaci* biotype B adult insects were inoculated near to the plants, for 24 h. After this period, the adult insects were removed from the plants and transferred to another rearing cage for 15 days until the nymphs reached the third instar. Third instar nymphs were used because of their immobility and ease of manipulation [14].

After that, the leaflets were detached from the infested plants and the nymphs present on the leaves were selected by marking with a marker pen (1 mm) next to the nymph [15]. Each treatment consisted of five leaflets of bean, each one containing 20 nymphs of third instar of *B. tabaci* Biotipe B. The leaflets were immersed in suspension containing 10 ml of distilled water + Tween® 20 (0.05%) + entomopathogenic fungus, at the concentration of 10^8 conidia/ml, for 1 min.

After complete drying, the leaflets were transferred individually to glass containers (10 ml) and the petioles immersed in agar-water solution (1.5%) to maintain leaf turgidity [16]. A control treatment was performed by immersing the bean leaflets in solution containing 10 ml of autoclaved water + Tween® 20 (0.05%). The containers containing the leaflets and nymphs of *B. tabaci* Biotipe B were transferred to a glass cage (65 × 30 × 45 cm), with the upper opening covered with a

Comment [MJ4]: Your references should be numbered in numerical order. Therefore, this should not be your 13th ref, but your 8th.

Comment [MJ5]: Which biocontrol agent was used?

Formatted: Font: 10 pt

Formatted: Font: 9 pt

Comment [MJ6]: This sentence is too long

Formatted: Font: Not Italic

Formatted: Font: 10 pt

Formatted: Font: 9 pt

Comment [MJ7]: Please indicate if other studies have been made on *Metarhizium rileyi*, *Beauveria bassiana* and *Lecanicillium muscarium* as biocontrol agent for the whitefly

Formatted: Font:

Comment [MJ8]: How did you identify the species?

Comment [MJ9]: What does that mean? Did you wait for the next generation?

Comment [MJ10]: Which locality?

Comment [MJ11]: Please be precise: where exactly did you inoculate the insects? On the plant? On the peat? Next to the pot?

Comment [MJ12]: Why do you keep the adults?

Comment [MJ13]: It is not clear. Do you do that so that adults can lay eggs on the plants?

Comment [MJ14]: What if you had extra nymphs on the same leave? Did you remove it manually?

"voil". The experiment was maintained in acclimatized room (25 ± 2 °C, $70 \pm 10\%$ RH and 12/12 h of photophase). The mortality of the nymphs was characterized by the flaccidity and whitish coloration of the insect tegument.

Pathogenicity bioassays with *B. tabaci* eggs

For the pathogenicity bioassays of the fungal isolates to eggs of *B. tabaci* biotype B, the same procedure used in the pathogenicity tests with nymphs was performed. After the oviposition period (24 h), the adults were removed from the infested plants and leaflets detached from the bean plants. The eggs were selected by lateral markings, as previously described. Each treatment consisted of five leaflets of bean, containing 20 eggs of *B. tabaci* biotype B (<48 h after oviposition). After marking, the leaflets were immersed in suspension containing 10 ml of distilled water + Tween® 20 (0.05%) + entomopathogenic fungus, in the concentration of 10^8 conidia/ml, for 1 min.

After drying, the bean leaflets containing the whitefly eggs were transferred individually to glass containers (10 ml) and the petioles immersed in agar-water solution (1.5%), in order to maintain the turgidity of the leaf [16]. Control treatment was performed immersing the leaflets in solution of 10 ml of distilled water + Tween® 20 (0.05%). The containers were maintained in acclimatized room (25 ± 2 °C, $70 \pm 10\%$ RH and 12/12h of photophase). Seven days after immersion of the leaves in the solution containing entomopathogenic fungus the eggs viability was evaluated. The unviability of eggs was verified by the observation of necrotic eggs and eggs that did not hatched, 5 days before the eggs hatching [17]. Both pathogenicity tests (nymphs and eggs) were performed in triplicate.

Comment [MJ15]: What do you mean? How do you know the eggs would hatch 5 days later?

Virulence of the entomopathogenic fungi isolates

For virulence tests, we performed Probit analysis and estimated the lethal concentration able to kill 50% of the population (LC_{50}) of the *B. tabaci* Biotype B of each isolate in order to evaluate the toxicity of the fungi isolates against *B. tabaci* biotype B and to estimate their dose-response. All the virulence tests were performed according to methodology described previously in the pathogenicity tests for *B. tabaci* biotype B nymphs and eggs. We considered only the isolates that caused mortality above 80% of the nymphs or eggs of *B. tabaci* biotype B. Six concentrations of each entomopathogenic fungus isolate was tested (10^3 , 10^4 , 10^5 , 10^6 , 10^7 and 10^8 conidia/ml) [18].

Comment [MJ16]: This should only be mentioned in the «statistical analysis» section

Comment [MJ17]: Why?

Statistical analysis

The results of the eggs and nymphs mortality of *B. tabaci* was previously corrected by the Abbott's formula [19]. The results expressed in percentages were used the arcsine square root transformation [20]. All the experiment was were performed in a completely randomized design, the results were submitted to ANOVA test and the means were compared by the Tukey test ($P < 0.05$). The LC_{50} was estimated by Probit analysis ($P < 0.05$). All analysis were performed in SAS package, version 9.1[21].

Comment [MJ18]: Check grammar

3. RESULTS AND DISCUSSION

All isolates evaluated were pathogenic to third instar nymphs and eggs of *B. tabaci* biotype B (Fig. 1 and Fig. 2). The isolates JAB07 of *B. bassiana* and LCMAP3790 of *L. muscarium* presented the greatest mortality rates, with mortality of $96.68 \pm 2.25\%$ and $97.74 \pm 1.56\%$ to third instar nymphs of *B. tabaci* Biotype B, respectively, differentiating of the IBCB66 isolate of *B. bassiana* that caused the lower mortality rate of $67.51 \pm 8.11\%$ ($F = 5.07$; $P < 0.0001$) (Fig. 1). The most of the isolates tested caused more than 80% of eggs unviability, except for *B. bassiana* isolate IBCB66, that caused only $27.54 \pm 2.67\%$ unviability ($F = 20.96$; $P < 0.0001$) (Fig 2).

Comment [MJ19]: This sentence is too long and difficult to follow

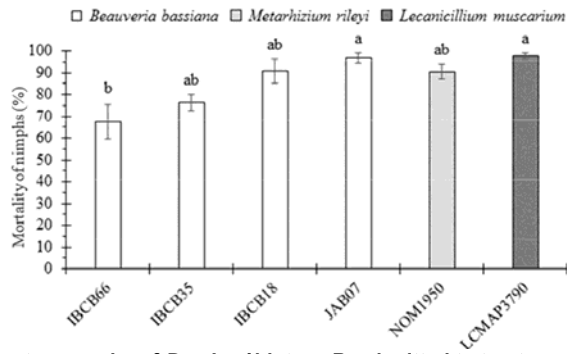


Figure 1. Mortality of third instar nymphs of *B. tabaci* biotype B submitted to treatments with entomopathogenic fungi isolates.

Columns followed by the same letter did not differ significantly from each other by the Tukey test ($P < 0.05$). The error bars represent the standard error of the mean (\pm SE).

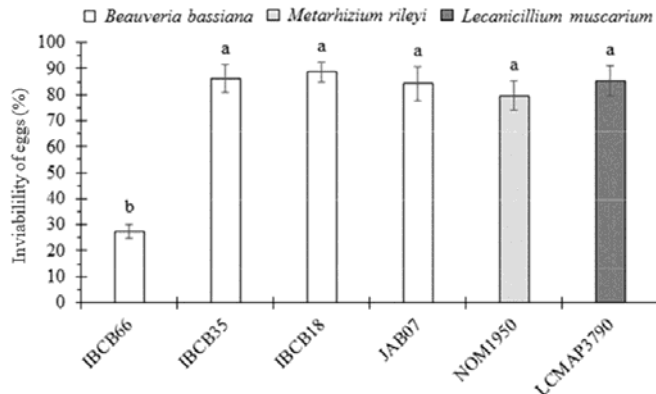


Figure 2. Unviability of eggs of *B. tabaci* biotype B submitted to treatments with entomopathogenic fungi isolates.

Columns followed by the same letter did not differ significantly from each other by the Tukey test ($P < 0.05$). The error bars represent the standard error of the mean (\pm SE).

For *B. tabaci* third instar nymphs, the LC_{50} varied between 0.006 and 0.217×10^3 conidia/ml, JAB07 isolate of *B. bassiana* was the most virulent among the isolate tested (Table 1). In relation to eggs viability results, JAB07 isolate also was the most virulent, with a LC_{50} estimate of 0.012×10^3 conidia/ml (Table 2). The best results aiming the *B. tabaci* Biotype B control are related with *B. bassiana* isolates, with mortality rates above 70% [16, 2]. Several studies confirm the potential of *B. bassiana* for the control of *B. tabaci* Biotype B, that represented by the greater amount of products formulated worldwide for this pest [18, 22].

In relation to *L. muscarium* species, even though the results were expressive in the present study, many studies have demonstrated the low efficiency in whitefly control, with mortality varying between 20 and 60% [12, 2]. It is important to highlight that the genus *Lecanicillium* has been used worldwide as bioinsecticide for the control of *B. tabaci* Biotype B, although with unsatisfactory results [10, 11, 12].

Table 1. Lethal concentration (LC_{50}) of entomopathogenic fungi isolates applied in third instar nymphs of *B. tabaci* biotype B after seven days of evaluation.

Fungi	Isolate	n	LC_{50} ($\times 10^3$ con.ml ⁻¹)	CI 95% ^{a,d}	Slope \pm SE _b	χ^2 ^c
<i>L. muscarium</i>	LCMAP3790	600	0.023	0.018 - 0.030	0.31 \pm 0.09	0.15
<i>B. bassiana</i>	IBCB18	600	0.084	0.062 - 0.115	0.23 \pm 0.05	49.81

Comment [MJ20]: What do you mean?

<i>B. bassiana</i>	JAB 07	600	0.006	0.002 - 0.012	0.20 ± 0.05	22.04
<i>M. rileyi</i>	NOM1950	600	0.217	0.098 - 0.428	0.14 ± 0.02	3.41

^a Confidence interval with fiducial limit of 95% probability.

^b Angular coefficient ± Standard error.

^c χ^2 = chi-square ($P < 0.05$).

^d 95% of probability confidence interval that does not overlap indicates statistically significant differences between LC_{50} values.

Table 2. Lethal concentration (LC_{50}) of entomopathogenic fungi isolates applied in eggs of *B. tabaci* biotype B after seven days of evaluation.

Fungi	Isolate	n	LC_{50} ($\times 10^3$ con.ml ⁻¹)	CI 95% ^{a,d}	Slope ± SE ^b	χ^2 ^c
<i>L. muscarium</i>	LCMAP3790	600	0.229	0.120 - 0.408	0.15 ± 0.02	10.59
<i>B. bassiana</i>	IBCB18	600	0.244	0.110 - 0.484	0.14 ± 0.02	1.78
<i>B. bassiana</i>	JAB 07	600	0.012	0.003 - 0.031	0.14 ± 0.02	8.76
<i>M. rileyi</i>	NOM1950	600	0.223	0.099 - 0.446	0.14 ± 0.02	0.98

^a Confidence interval with fiducial limit of 95% probability.

^b Angular coefficient ± Standard error.

^c χ^2 = Chi-square ($P < 0.05$).

^d 95% of probability confidence interval that does not overlap indicates statistically significant differences between LC_{50} values.

Most of the isolates of the entomopathogenic fungi tested caused high eggs unviability of *B. tabaci* Biotype B, especially *B. bassiana* JAB07 isolate, being the most virulent among the entomopathogenic fungi evaluated, with LC_{50} estimated of 0.012 and 0.006×10^3 conidia/ml for eggs and nymphs of *B. tabaci* Biotype B, respectively. However, only the IBCB66 isolate of *B. bassiana* did not present ovicidal activity [17, 23].

NOM1950 isolate of *M. rileyi* presented high mortality rate and activity ovicidal of *B. tabaci* biotype B. This entomopathogen is considered an important biological control agent against insect species of the Noctuidae family (Lepidoptera) [24, 25, 26, 27, 28]. Additionally, this study evidenced high control efficiency of this entomopathogenic fungus against whitefly eggs and nymphs, demonstrating its potential as biological control agent of this important insect pest.

In conclusion, JAB07 of *B. bassiana* and LCMAP3790 isolates from *L. muscarium* presented the best results for *B. tabaci* Biotype B control. Moreover, the isolate JAB07 was the most virulent against eggs and nymphs, indicating its potential for further production of formulated products. Initial tests to select isolates of entomopathogenic fungi of high virulence are fundamental to increase the efficiency of the integrated program management of this pest. However, other biocontrol's strategies should be considered for effective and more sustainable control of the whitefly. Furthermore, future studies under field conditions are required, making it possible to analyze the real efficiency of these isolates, in addition to questions related to persistence and their compatibility with agrochemicals.

4. CONCLUSION

The JAB07 and IBCB18 isolates of *B. bassiana* and LCMAP3790 isolate of *L. muscarium* were that caused higher mortality of nymphs and eggs of *B. tabaci* biotype B.

JAB07 isolate of *B. bassiana* was the most virulent for eggs and nymphs of *B. tabaci* biotype B, obtaining LC_{50} estimated to 0.012 and 0.006×10^3 conidia/ml, respectively, and can be indicated for further field tests for the control of *B. tabaci* biotype B.

Comment [MJ21]: Redundant with the last paragraph of your discussion

REFERENCES

- Oliveira M R V, Henneberry T E, Anderson P. History, current status, and collaborative research projects for *B. tabaci*. Crop Protection, v. 20, p. 709-723, 2001.

2. Mascarin G M, Kobori N N, Quintela E D, Delalibera J I. The virulence of entomopathogenic fungi against *Bemisia tabaci* biotype B (Hemiptera: Aleyrodidae) and their conidial production using solid substrate fermentation. *Biological Control*, v. 66, p. 209-218, 2013.
3. Oliveira C M, Auad A M, Mendes S M, Frizzas M R. Economic impact of exotic insect pests in Brazilian agriculture. *Journal of Applied Entomology*, v. 137, p. 1–15, 2013.
4. Byrne D, Miller W B. Carbohydrate and amino acid composition of phloem sap and honeydew produced by *Bemisia tabaci*. *Journal of Insect Physiology*, v. 36, p. 433-439, 1990.
5. Sinisterra X H, McKenzie C L, Hunter W B, Powell C A, Shatters J R G. Differential transcriptional activity of plant-pathogenic begomoviruses in their whitefly vector (*Bemisia tabaci*, Gennadius: Hemiptera Aleyrodidae). *Journal of General Virology*, v. 86, p. 1525-1532, 2005.
6. Barbosa J C, Barreto S D S, Inoue-Nagata A K, Rezende J A M. Characterization and experimental host range of a Brazilian tomato isolate of Tomato severe rugose virus. *Journal of Phytopathology*, v. 159, p. 644-646, 2011.
7. Basit M, Saeed S, Saleem M A, Denholm I, Shah M. Detection of resistance, cross-resistance, and stability of resistance to new chemistry insecticides in *Bemisia tabaci* (Homoptera: Aleyrodidae). *Journal of Economic Entomology*, v. 106, p. 1414–1422, 2013.
8. Shah M M R, Zhang S, Liu T. Whitefly, Host Plant and Parasitoid: A Review on Their Interactions, *Asian Journal of Applied Science and Engineering*, v. 4, p. 48-61, 2015.
9. Tian J, Diao H, Liang L, Hao C, Arthurs S, Ma R. Pathogenicity of *Isaria fumosorosea* to *Bemisia tabaci*, with some observations on the fungal infection process and host immune response. *Journal of Invertebrate Pathology*, v. 130, p. 147-153, 2015.
10. Faria M R, Wraight S P. Mycoinsecticides and mycoacaricides: a comprehensive list with worldwide coverage and international classification of formulation types. *Biological Control*, v. 43, p. 237–256, 2007.
11. Goettel M S, Koike M, Kim J J, Aiuchi D, Shinya R, Brodeur J. Potential of *Lecanicillium* spp. for management of insects, nematodes and plant diseases. *Journal of Invertebrate Pathology*, v. 98, p. 256–261, 2008.
12. Cuthbertson A G S, Blackburn L F, Northing P, Luo W, Cannon R J C, Walters K F. A. Leaf dipping as an environmental screening measure to test chemical efficacy against *Bemisia tabaci* on poinsettia plants. *International Journal of Environmental Science & Technology*, v. 6, p. 347–352, 2009.
13. Pereira R A S, Menezes-Júnior A O, SUGAWARA L M. Criação da mosca branca *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) em folhas enraizadas de soja (*Glycine max* (L.) Merrill.). *Semina: Ciências Agrárias*, v. 17, n. 1, p. 45-48, 1996.
14. James R R, Buckner J S, Freeman T P. Cuticular lipids and silverleaf whitefly stage effect conidial germination of *Beauveria bassiana* and *Paecilomyces fumosoroseus*. *Journal of Invertebrate Pathology*, v. 84, p. 67-74, 2003.
15. Vicentini S, Faria M, de Oliveira M R. Screening of *Beauveria bassiana* (Deuteromycotina: Hyphomycetes) isolates against nymphs of *Bemisia tabaci* (Genn.) Biotype B (Hemiptera: Aleyrodidae) with description of a new bioassay method. *Neotropical Entomology*, v. 30, p. 97-103, 2001.
16. Potrich M, Neves P M O J, Angeli L F, de Gouvea A, Roman J C. Virulência de fungos entomopatogênicos a ninfas de *Bemisia tabaci* (Genn.) (Hemiptera: Aleyrodidae). *Semina: Ciências Agrárias*, v. 32, p. 1783-1792, 2011.
17. Gindin G, Geschtovt N U, Raccah B, Barash I. Pathogenicity of *Verticillium lecanii* to different developmental stages of the silverleaf whitefly, *Bemisia argentifolii*. *Phytoparasitica*, v. 28, p. 229-239, 2000.
18. Quesada-Moraga E E A A, Maranhao E A A, Valverde-García P, Santiago-Álvarez C. Selection of *Beauveria bassiana* isolates for control of the whiteflies *Bemisia tabaci* and *Trialeurodes vaporariorum* on the basis of their virulence, thermal requirements, and toxicogenic activity. *Biological Control*, v. 36, p. 274-287, 2006.
19. Abbott W S. A method of computing the effectiveness of an insecticide. *Journal of Economic Entomology*, v. 18, p. 265-267, 1925.
20. Sokal R R, ROHLF F J. *Biometry*. Freeman, New York, 887 p. Warton D I, Hui F K C. 2011. The arcsine is asinine: the analysis of proportions in ecology. *Ecology* 92:3–10, 1995.
21. SAS Institute. *User's guide: statistics, version 9.1*. SAS Institute Inc. Cary, NC, 2002.
22. Cabanillas E H, Jones A W. Effects of Temperature and culture media on vegetative growth of an entomopathogenic fungus *Isaria* sp. (Hypocreales: Clavicipitaceae) naturally affecting the whitefly, *Bemisia tabaci* in Texas. *Mycopathologia*, v. 167, p. 263-271, 2009.
23. Espinel C, TORRES L. Effect of entomopathogenic fungi over development stages of *Bemisia tabaci* (Hemiptera: Aleyrodidae). *Revista Colombiana de Entomologia*, v. 35, p. 18-21, 2009.
24. Ignoffo C M. The fungus *Nomureae rileyi* as a microbial insecticide. In: Burges H D. *Microbial Control Pests of Pests and plant diseases, 1970-1980*. New York, London: Academic Press, 1981, p. 523-538.
25. Devi P V, Prasad Y G, Chowdary D A, Ra L M, Balakrishnan K. Identification of virulent isolates of the entomopathogenic fungus *Nomureae rileyi* (F) Samson for the management of *Helicoverpa armigera* and *Spodoptera litura*. *Mycopathologia*, v. 156, p. 365-373, 2003.

26. Sosa-Gómez D R, Delpin K E, Moscardi F, Nozaki M D H. The impact of fungicides on *Nomureae rileyi* (Farlow) Sanson epizootics and on populations of *Anticarsia gemmatalis* Hubner (Lepidoptera: Noctuidade) on soybean, Neotropical Entomology, v. 32, p. 287-291, 2003.
27. Sun B D, Yu H Y, Chen A J, Liu X Z. Insect-associated fungi in soils of field crops and orchards, Crop Protection, v. 27, p. 1421-1426, 2008.
28. Duarte R T, Gonçalves K C, Espinosa D J L, Moreira L F, De Bortoli S A. Humber, R. A., & Polanczyk, R. A. Potential of entomopathogenic fungi as biological control agents of diamondback moth (Lepidoptera: Plutellidae) and compatibility with chemical insecticides. Journal of Economic Entomology, v. 109, n. 2, p. 594-601, 2016.

UNDER PEER REVIEW